"Development and Validation of RP-HPLC method, HPTLC method and UV- Spectrophotometric simultaneous equation method of Pioglitazone, Glimepiride and Metformin in combined tablet dosage form"

"UV Spectrophotometric simultaneous equation method, UV Spectrophotometric absorbance ratio method for Nebivolol and Hydrochlorothiazide, Lumifantrine and Artemether in combined tablet dosage form"

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CERTIFICATE

This is to certify that the Dissertation entitled **"Development and Validation of RP-HPLC method, HPTLC method and UV-Spectrophotometric simultaneous equation method of Pioglitazone, Glimepiride and Metformin in combined tablet dosage form", "UV Spectrophotometric simultaneous equation method, UV Spectrophotometric absorbance ratio method for Nebivolol and Hydrochlorothiazide, Lumifantrine and Artemether in combined tablet dosage form"** by **Mr.S.U.WAHAB** in the Department of Pharmaceutical Chemistry, Madurai Medical College, Madurai – 625 020, in partial fulfillment of the requirements for the Degree of Master of Pharmacy in Pharmaceutical Chemistry under my guidance and supervision during the academic year 2007-2008.

This dissertation is forwarded to The Controller of Examination, The Tamil Nadu Dr.MGR Medical University, Chennai.

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Date:

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I hereby dedicate this little piece of work to Almighty.

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INDEX

INTRODUCTION

Analytical techniques hold the key to the design, development standardization and quality control of medical products.

They are equally important in pharmacokinetics and in drug metabolism studies, both of which are fundamental to the assessment of bioavailability and the duration of clinical response.

The various analytical techniques available are,

1. Gravimetric Analysis
2. Titrimetric Analysis
3. Volumetry
4. Electrical Methods
5. Optical Methods

Spectroscopy:

It is the measurement and interpretation of electromagnetic radiations absorbed or emitted when the molecules or atoms or ions of the sample undergo transition from one energy state (Ground state) to another (excited state).

It is two types,

1. Absorption Spectroscopy:

Where absorption of electro magnetic radiation (EMR) takes place.

(eg.) Colorimetry, UV spectroscopy, IR spectroscopy, etc.

2. Emission Spectroscopy:

Where emission of radiation is being studied.

(eg.) Fluorimetry, Flame Photometry.

Assay of Substances in Multi Component Samples:

There are

1. Simultaneous equation method
2. Absorbance ratio method

Simultaneous Equation Method:

If a sample contains two absorbing drugs (X and Y) each of which absorbes at the λ_{max} of the other. It may be possible to determine the quantity of both drugs by the technique of simultaneous equation.

Absorbance Ration Method:

This method depends on the property of a substance which obeys Beer's law at all wavelengths, the ration of absorbance at any two wavelengths is a constant value independent of concentration or path length. Two different dilutions of the same sample give the same absorbance ratio; this ratio is referred to as a 'Q' value.

CHROMATOGRAPHY

Introduction:

Modern pharmaceutical formulations are complex mixtures containing one or more therapeutically active ingredients. The most powerful techniques available to the analyst for the separation of there mixtures, a group of highly efficient methods which are collectively called as chromatography.

It's a group of technique which works on the principle of separation of components of a mixture into individual components, depending on their affinities for the solutes between two immiscible phases.

High Performance Liquid Chromatography:

The technique is based on the same modes of separation as classical column chromatography. i.e. adsorption, partition (including reversed-phase partition), ion exchange and gel permeation.

According to mode of separation, the stationary phase is polar and the mobile phase is non-polar is called as **normal phase high performance liquid chromatography**.

The stationary phase is non-polar and the mobile phase is polar is called as **reverse phase high performance liquid chromatography**.

AIM OF PRESENT WORK

The newer antiviral drugs in combined dosage form are selected for the analysis by high performance liquid chromatography method.

It should be more accurate, simple, rapid, efficient than other methods of analytical techniques.

Since, the combined dosage formulation can not be easily separated by other analytical technique like UV and other titrimetric analysis.

So, that I shall select this HPLC for the separation and estimation of newer anti viral combined dosage forms. It will be simple, rapid, efficient and reliable for the analysis of the drugs in combined dosage form.

Newer Combined Dosage Form of Anti Viral Drugs:

1. Efavirence + Zidovudine + Lamivudine
2. Lopinavir + Ritonavir
3. Lamivudine + Zidovudine
4. Stavudine + Lamivudine
5. Nevirapine + Stavudine + Lamivudine
6. Nevirapine + Zidovudine + Lamivudine

DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF NEWER ANTI VIRAL DRUGS IN COMBINED DOSAGE FORM

Method Development and Optimization:

Selection of Wavelength:

The known concentration of a Newer Anti Viral Drugs in Combined Dosage Form is taken dissolved in methanol. To fix the wavelength in the region of 200 -400nm and the wavelength which we select will be no fronting and tailing and the peak area is also found be minimum.

Selection of Mode of Operation:

According to nature of drugs (polar / non-polar) the HPLC method will be selected. (Polar-RPHPLC, Non-polar-Normal phase HPLC)

Selection and Standardization of Mobile Phase:

The mobile phase will be selected accordingly which will produce required adequate resolution of drug peaks in the chromatogram.

Selection of Solvent System:

The peak of combined drug is well resolved with solvent system and buffer.

Selection of Flow Rate:

The flow rate for combined drug is selected should not show any fronting and tailing.

Preparation of Mobile Phase:

The selected buffer and solvent system is mixed together in appropriate ratio and degass it.

Diluent:

Mobile phase used as diluent.

Determination of Retention Time:

The time at which the components are eluted to produce a peak.

Procedure:

Standard Solution of Drugs:

Accurately 100mg of standard drugs in combined dosage form was taken 100ml volumetric flask and the volume was adjusted to 100ml with mobile phase. From this 5ml is diluted to 50ml with mobile phase. 20µl of the solution injected and chromatogram is obtained.

Preparation of Sample Solution:

Twenty tablets are weighed and powdered. Average weight is calculated. From this equivalent to 100mg is taken into 100ml volumetric flask. The powder was first dissolved in 20ml of mobile phase and sonicated and finally the volume was adjusted to 100ml with mobile phase. From this solution 5ml was transferred to 50ml volumetric flask and volume was adjusted to 50ml with mobile phase. 20µl of the solution injected and chromatogram is obtained.

The amount of drugs present in the combined dosage formulation is calculated by comparing the peak area of standard.

Amount of drug present in the tablet =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \frac{\text{Potency}}{100} \times \text{Average Weight}$$

$$\text{Percentage Purity} = \frac{\text{Amount present}}{\text{Label Claim}} \times 100$$

VALIDATION

Method validation is the process of proving that an analytical method is acceptable for its intended purpose. For pharmaceutical methods, guidelines from the United States Pharmacopeia (USP) and International Conference on Harmonization (ICH). In general, methods for regulatory submission must include studies on specificity, linearity, accuracy, precision, range, detection limit, quantitation limit, and robustness.

Design of Experiments:

Typical analytical parameters used in assay validation are,

- Specificity
- Linearity and range
- Limit of quantification
- Limit of detection
- Accuracy
- Precision
    - ⇒ System precision
    - ⇒ Method precision
- Robustness
- Ruggedness
- System suitability studies
    - ⇒ Resolution
    - ⇒ Number of theoretical plates

⇒ The tailing factor

SPECIFICITY

The specificity of an analytical method is its ability to measure accurately the analytes in the presence of compounds that may be expected to be presented to be present in the sample matrix.

Determination:

The specificity of the analytical method was determined by injecting the diluents or excipients solution under the same experimental conditions as the assay.

Procedure:

1. The excipients of dissolved in mobile phase from this 20µl of the solution injected and chromatogram is obtained.
2. The excipients along with standard drugs are dissolved in mobile phase from this 20µl of the solution injected and chromatogram is obtained.

LINEARITY AND RANGE

Linearity of an analytical method is verified that the sample solutions are in a concentration range where analyte response is directly proportional to the concentration.

Determination:

The linearity of analytical method was determined by preparing a linear concentration of standard mixture solution. 20µl of the solution injected and chromatogram is obtained. Area was plotted graphically as a function of drug concentration to get linear curve.

Range:

It is the interval of lowest concentration to largest concentration to give linear response.

ACCURACY

The accuracy of an analytical method is the closeness of the results obtained by that method to the true value. Accuracy may often by expressed as percent recovery by the assay of known added amount of analyte.

Determination:

The accuracy of the analytical method was determined by applying the method to the analysed samples to which known amounts of analyte had been added. The accuracy was calculated from the test results as the percentage of analyte recovered by the assay.

Acceptance Criteria:

Percentage recovery should be within 98-102% at each concentration over the range of 80-120%.

Procedure:

Mixed standard stock solution 2ml and sample stock solution 2ml were mixed together in 50ml volumetric flask and the volume was made upto 50ml with mobile phase to get 80% range. Similarly 100% (2ml standard + 3ml sample) and 120% (2lm standard + 4ml sample). 20µl of the solution injected 3 times and chromatogram is obtained.

PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample.

Determination:

The precision of the analytical method was determined by assaying sufficient number of sample and relative standard deviation was calculated.

Acceptance Criteria:

The relative standard deviation should be with in 2%.

System Precision:

The system precision was evaluated by measuring 6 successive injection of 20µl of standard solution. The peak response were measured from the chromatogram.

Method Precision:

The method precision was evaluated by measuring 6 successive injection of 20µl of sample solution. The peak response were measured from the chromatogram.

LIMIT OF DETECTION (LOD)

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conditions. The detection limit is usually expressed as the concentration of analyte.

$$LOD = \frac{3.3 \times \sigma}{m}$$

σ - Standard deviation of the response

m - Slope of the calibration curve

LIMIT OF QUANTITATION (LOQ)

The Quantitation limit of an analytical procedure is the lowest amount of analyte which can be Quantitatively determined with suitable Precision and Accuracy.

$$LOQ = \frac{10 \times \sigma}{m}$$

σ - Standard deviation of the response

m - Slope of the calibration curve

RUGGEDNESS

The ruggedness of an analytical method is degree of reproducibility of test result obtained by the analysis of the same sample under a variety of normal test condition, such as different laboratories, different analyst, different instruments, different lots of reagents, different elapsed assay times, different assay temperature, different days, etc.

ROBUSTNESS

Robustness of an analytical method is measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Determination:

The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but were still within the specified parameter of the assay for example change in physical parameters like flow rate, pH and lambda max.

SYSTEM SUITABILITY TESTING

It is an integral part of many analytical procedure. The tests of based on the concept that the equipment, electronics, analytical operations and sample to be analyzed constitute an integral system that can be evaluated as such.

The parameters are,

1. Resolution:

$$R = \frac{2\,(Rt_2 - Rt_1)}{W_2 + W_1}$$

Where,

Rt_1 and Rt_2 = Retention time of two component

W_1 and W_2 = The respective peak width

Acceptance Criteria:

It always >1.

2. Tailing Factor:

Fronting is due to saturation of stationary phase and can be avoided by using less quantity of sample. Tailing is due to more active adsorption sites and can be eliminated by support pretreatment, more polar mobile phased increasing the amount of liquid phase.

$$T = \frac{Y_x}{2A}$$

Where,

T - Tailing Factor

Y_x - The width of the peak at one twentieth of the peak height

A - The distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one twentieth of the peak height.

3. Number of Theoretical Plates:

$$N = 5.54 \times \left(\frac{Rt}{W_{h/2}} \right)^2$$

Rt - Retention Time

$W_{h/2}$ - Width of peak at half height

I shall complete this research project work with in Three years

DRUG PROFILE

Drug Name	:	Zidovudine
Structure		
Chemical Name	:	3’-Azido-3’ deoxythymidine
Molecular Formula	:	$C_{10}H_{13}N_5O_4$
Molecular Weight	:	267.2
Description	:	A white to yellowish crystalline powder
Solubility	:	Sparingly soluble in water freely soluble in methanol, hot water ethanol
Therapeutic Category	:	Antiviral / Nucleoside reverse transcriptase inhibitor

DRUG PROFILE

Drug Name	:	Stavudine
Structure		
Chemical Name	:	2’3’-Didehydro-3-deoxy-thymidine
Molecular Formula	:	$C_{10}H_{12}N_2O_4$
Molecular Weight	:	224.2
Description	:	White to off – white crystalline powder
Solubility	:	Freely soluble in methanol, distilled water
Therapeutic Category	:	Antiviral / Nucleoside reverse transcriptase inhibitor

DRUG PROFILE

Drug Name	:	Lamivudine
Structure		
Chemical Name	:	(2R Cis) 4 Amino-1-[2-(hydroxymethyl) – 1,3 oxathiolan -5-y;] -2 (lH)- pyrimidinone
Molecular Formula	:	$C_8H_{11}N_3O_3S$
Molecular Weight	:	229.3
Description	:	White to off – whit crystals
Solubility	:	Freely soluble in distilled water, ethanol slightly soluble in ethyl acetate
Therapeutic Category	:	Antiviral / Nucleoside reverse transcriptase inhibitor

DRUG PROFILE

Drug Name	:	Nevirapine
Structure		
Chemical Name	:	1-cyclopropyl-5, 11-dihydro-4-methyl-6H – dipyrido [3,2-b:2', 3'-e][1,4] diazepin-6-one
Molecular Formula	:	$C_{15}H_{14}N_4O$
Molecular Weight	:	266.298
Description	:	White to off – white crystalline powder
Solubility	:	Freely soluble in methanol, distilled water
Therapeutic Category	:	Antiviral / Nucleoside reverse transcriptase inhibitor

DRUG PROFILE

Drug Name	:	Efavirenz
Structure		
Chemical Name	:	8-chloro-5-(2-cyclopropylethynyl)-5-(trifluoromethyl)-4-oxa-2-azabicyclo [4.4.0] deca-7,9,11-trien-3-one
Molecular Formula	:	$C_{14}H_9ClF_3NO_2$
Molecular Weight	:	315.675
Description	:	White to off – white crystalline powder
Solubility	:	Freely soluble in methanol, distilled water
Therapeutic Category	:	Antiviral / Nucleoside reverse transcriptase inhibitor

REVIEW OF LITERATURE

Asha Thomas, et al, Estimated simultaneous equation method, derivative spectroscopy method and AUC method have been developed for the simultaneous estimation of metformin, pioglitazone and glimeprider in their combined dosage form.

Radha Krishna T.Raods et al, High performance liquid chromatographic and micellar electrokinetic chromatographic methods have been developed for the determination of pioghitazone hydrochloride in bulk and acetonitrle: Buffer in the ration of 50:50, as mobile phase. The pH is adjusted to 6 using O-phosphoric acid.

Sripalakit et al, High performance liquid chromatography with ultra violet detection (269nm) was developed for the determination of pioglitazone in human plasma. Rosiglitazone was used as an internal standard. The separation was achieved with a RP-HPLC, a mobile phase of methanol: Acetonitrile: Buffer (pH 2.6, 40:12:48 v/v/v) with flow rate of 1.2ml/ min. The linearity range of 50-2000ng/ml and the lower limit of quantification was 50ng/ml.

Zhang.L. Tian.Y. et al, A selective and sensitive HPLC – electroscopy ionization tandem MS method for simultaneous determination of metformin and rosiglitazone in human plasma using phenformin as internal standard has been first developed and validated. The separation was

achieved by using a mobile phase methanol : 30mn ammonium acetates pH 5.0 (80:20 v/v) delivered at 0.2ml/min.

D.Bhavesh et al, Estimated a simple and rapid HPLC assay method for the stimation of metformin in human plasma was developed and validated. The separation was achieved with a cationic exchange coloumn with mobile phase of methanol : pot dihydrogen ortho phosphate buffer (0.1m, pH 3.5) mixture 46:54% v/v. Detection was by UV detector at 236nm. The response was linear over a range of 30-5000ng/ml.

Dr.Harising Gowr et al, Performed a rapid and simple simultaneous estimation of metformin and gliclazide in combined dosage forms by simultaneous equation at two wavelengths corresponding to 226.3 and 233.2nm. Both the drugs are obey Beer's law in the concentration ranges employed for this method. The result of analysis have been validated statistically and by recovery studies.

Madhira B, Shankar et al, Performed spectrophotometric analysis of pioglitazone hydrochloride and metformin hydrochloride in combined dosage forms were developed using second derivative spectrophotometry and RP-HPLC. In the LC method, analysis was performed on a C_{18} column, using the mobile phase acetonitrile – water – acetic acid (75:25:0.3) adjusted to pH 5.5 with liquid ammonia, at a flow rate of 0.5ml/min. The retention times were 8.5min for PIO, 16min to MET. The concentration range of 4-

20µg/ml for P_{10} and MET. The methods are validated results were compared statistically.

Bhaskar Laxmanrao Klte et al, Performed simultaneous determination of metformin and glimepride in pharmaceutical dosage form by RP-HPLC. Under the developed conditions, good separation of the analytes was achieved in short analysis time. The method is validated and shown to be linear in the range of 25-150µg/ml for metformin and 0.1µg/ml to 0.6µg/ml for glimepride.

A.Ghassempour et al, Estimated simultaneous determination of two antidiabetic drugs metformin and glyburide in pharmaceutical tablet formulation were investigated. Normal phase thirl layer chromatography (silica gel 60 F_{254}) was used as stationary phase and water: methanol: ammonium sulfate (2:1:0.5w/v) as mobile phase to determine two pharmaceutically active ingredients, in three different formulations. This system gave a good resolution for metformin Rf 0.43 $\pm$ 0.01 and glyburide Rf 0.64 $\pm$ 0.02. The absorbance mode was selected at 237nm. The limit of detection and quantification were 25.24 and 84.12ng/spot for metformin and 12.26 and 40.86ng/spot for glyburide.

Annapurna M, developed spectrophotometric estimation of metformin hydrochloride in pharmaceutical dosage forms.

Cum-Gangding et al Developed a selective sensitive high performance liquid chromatography for simultaneous determination of

metformin and glipizide in human plasma, has been developed for simultaneous quantification of metformin and glipizide in human plasma phenformin anf gliclazide were used as internal standards for metformin and glipizide respectively. The MS detection was performed in multiple reaction monitoring mode. The method was validated in the concentration ranges of 0.02-4µg/ml and 0.004-0.8µg/ml for metformin and glipizide respectively.

Shankar, Modi et al, Performed spectrophotometric and liquid chromatographic methods for simultaneous estimation of pioglitazone and metformin hydrochloride in tablet formulation. The pioglitazone and metformin in combined preparation were quantified using the second derivative responses at 227.5nm for pioglitazone and 257.25nm for metformin in spectra of their solutions in a minture of methanol and acetonitrile. The calibration cusves were linear in the concentration range of 8-40µg/ml for PID and 4-12µg/ml for MET.

The second method LC was performed on Hypersil ODS C_{18} column with 5 micron particle size using the mobile phase acetonitrile: water: acetic acid (75+25+0.3) adhusted to pH 5.5 with liquor ammonia, and flow rate 0.5nl/min. Measurement was made at a wavelength of 230nm.

M.Vasudevan et al Developed a simple precise and accurate high performance liquid chromatography for the simultaneous estimation of metformin, gliclazide and glizide present in multicomponent dosage forms. A mobile phase composed of acetonitrile water containing camphor

sulphonic acid (adjusted to pH 7 using N/10 sodium hydroxide; 75mm) at a a flow rate 1ml/min used for the separation. Detection was carried out at 225nm, tolbutamide was used as internal standard validation of the developed HPLC method was carried out.

Mistri HN et al, Performed liquid chromatography tandem mass spectrornetry method for simultaneous quantitation of antidiabeitc drugs metformin and glyburide in human plasma using glimepride as internal standard. Metformin, glyburide and internal standard were chromatographed on RP-C_{18} column. Quantization was performed on a triple quadrcepole mass spectrometer employing electroscopy ionization technique. The total chromatographic run time was 3.5min and calibration curves were linear over the 20-2500ng/ml for metformin and 5-500ng/ml for glyburide. The method was calidated for selectively, recovery specificity linearity precision and stability studies.

Zhang Z, Chen Y et al Developed a selective and sensitive high performance liquid chromatography for simultaneous estimation of metformin and rosiglitazone in human plasma. The mobile phase was methanol : Ammonium acetate (80:20) pH adjusted to 5 used and the retention time delivered at 0.2ml/min.

Anna berecka et al, Performed a new simple, rapid and stability indicating high performance thin layer chromatographic method has been developed and validated for the determination of rosiglitazone in tablets.

Analysis was performed on silica gel $60F_{254}$ plates in horizontal chambers with chloroform : wthyl acetate : 25% ammonium hydroxide (5:5:0.1) The calibration plots were constructed in the range of 0.2-1µg/10µl.

Ramesh T.Sane et al, Estimated a high performance thin layer chromatographic method has been developed for the determination of rosiglitazone in pharmaceutical preparations. This method uses silica gel $60F_{254}$ as the stationary phase, ethyl acetate : Toluene : Methanol (45:55:1v/v) as mobile phase. Detection was performed at 242nm and pioglitazone hydrochloride was used as internal standard. The method was validated to determine its accuracy and precision.

Kolte BL, et al develop a simple, rapid, and precise method is developed for the quantitative simultaneous determination of metformin and pioglitazone in a combined pharmaceutical dosage form. Separation is achieved with a zorbax XDB C(18), 15cm analytical column using buffer acetonitirle (66:34, v/v) of pH 7.1, adjusted with orthophosphoric acid as the mobile phase. The buffer used in the mobile phase contains 10mm dospdium hydrogen phosphate and 5mm sodium dodecyl sulphate in double-distilled water. The instrumental settings are flow rate of 1ml/min, column temperature at 40 degrees C, and detector wavelength of 226nm. The correlation coefficients for metformin an dpioglitazone are 0.9991 and 0.9999, respectively. The relative standard deviations for six replicate measurements in two sets of each drug in the tablets are always less than 2%.

Radhakrishna T, et al, performed high performance liquid chromatographic (HPLC) and Micellar Electrokinetic Chromatographic (MEKC) methods have been developed for the determination of pioglitazone, a new englycemic antidiabetic agent. The MEKC method was compared with HPLC method using a 5 microm symmetry C18 column (250 x 4.6mm id.) eluted with a mobile phase consisting of a mixture of 50% (v/v) acetonitrile and 10mm potassium dihydrogen phosphate buffer, adjusting the pH to 6.0 with 0.1m KOH. The HPLC method is capable of detecting all process related compounds, which may be present at trace levels in finished products. Both methods were fully validated and a comparison was made.

Khan MA, et al developed five impurities in glimepiride drug substance were detected and quantified using a simple isocratic reverse phase HPLC method. For the identification and characterization purpose these impurities were isolated from a crude reaction mixture of glimepiride using a normal phase HPLC system. The chromatographic separation was achieved on a phenomenex luna C8 (2) 100A, 5 microm, 250mm x 4.6mm using a mobile phase consisting of phosphate buffer (pH 7.0) acetonitrile – tetrahydrofurna (73:18:09, v/v/v) with UV detection at 228nm and a flow rate of 1ml/min. the column temperature was maintained at 35 degrees C through out the analysis. The method has been validated as per international guidelines on method validation and can be used for the routine quality control analysis of glimepiride as active pharmaceutical ingredient (API).

Dubey A; Shukla IC, et al estimated a new HPLC method for the determination of glipizide and metformin hydrochloride in combination. The method is based on reverse phase liquid chromatography using C18 column and a suitable mobile phase. The detection is done at 225nm. The flow rate is adjusted at 1.0ml/min. and the linearity is established.

Zarapkar SS; Salunkhe BB, et al performed a high performance thin layer chromatographic method for the determination of glipizide in tablets is described.

Pak J Pharm Sci et al, performed the reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed to quantify metformin hydrochloride (MFCI) in raw material and pharmaceutical formulations using C18 analytical reverse phase column. Diazepam was used as an internal standard. Mobile phase consisted of methanol water (30:70 v/v), pumped at a flow rate of 0.5ml/min at ambient temperature and the retention time was about 4.4min with symmetrical peaks. (MFCI) was detected by ultraviolet absorbance at 233nm with no interference of commonly used excipients. The method was linear over the concentration range 0.312-5µg/ml (R2 = 0.9995). The limit of detection of metformin was 0.1µg/ml and the limit of quantitation was 0.3µg/ml. The results obtained showed a good agreement with the declared contents in case of pharmaceutical formulations. The proposed method is rapid, accurate,

economical and selective and it may be used for the quantitative analysis of metformin.

R.T.Sane, S.N.Menon, et al, performed a rapid and accurate HPLC method has been developed for simultaneous determination of pioglitazone and glimepiride. Chromatographic separation of the two pharmaceuticals was performed on a Cosmosil C18 column (150mm x 4.6mm, 5µm) with a 45:35:20 (v/v) mixture of 0.01m triammonium citrate (pH adjusted to 6.95 with orthophosphoric acid), acetonitrile, and methanol as mobile phase, at 228nm. Separation was complete in less than 10min. The method was validated for linearity, accuracy, precision, limit of quantitation, and robustness. Linearity over the ranges 2.50-30.00µg/ml^{-1} for pioglitazone and 0.10-10.00µg/ml^{-1} for glimepiride.

Radhakrishna T, Sreenivas Rao D, et al, worked a high performance liquid chromatographic (HPLC) and Micellar Electrokinetic Chromatographic (MEKC) methods have been developed for the determination of pioglitazone, a new englycemic antidiabetic agent. 20% acetonitrile (v/v) in 20mm sodium borate buffer pH 9.3 containing 50mm sodium dodecyl sulphate (SDS). HPLC method using a 5 microm symmetry C18 column (250 x 4.6mm i.d.) eluted with a mobile phase consisting of a mixture of 50% (v/v) acetonitrile and 10mm potassium dihydrogen phosphate buffer, adjusting the pH to 6.0 with 0.1m KOH. The results confirm that the methods are highly suitable for its intended purpose.

Kolte B.L. Raut B.B., et al performed a simple, rapid, and precise method is developed for the quantitative simultaneous determination of metformin and pioglitazone in a combined pharmaceutical dosage form. Separation is achieved with a Zorbax XDB C18, 15cm analytical column using buffer acetonitrile (66:34, v/v) of pH 7.1, adjusted with orthophosphoric acid as the mobile phase. The buffer used in the mobile phase contains 10mm disodium hydrogen phosphate and 5mm sodium dodecyl sulphate in double distilled water. The instrumental settings are flow rate of 1ml/min, column temperature at 40^0C, and detector wavelength of 226nm. The internal standard method is used for the quantitation of the ingredients of this combination. The method is validated and shown to be linear for metformin and pioglitazone are 0.9991 and 0.9999, respectively. The relative standard deviations for six replicate measurements in two sets of each drug in the tablets are always less than 2%.

Lin Z.J, Ji W, Desai-Krieger D, Shum L. et al, performed a liquid chromatography / tandem mass spectrometry (LC-MS/MS) method was developed and validated for the simultaneous determination of pioglitazone (PIO) and its two metabolites. The compounds were eluted isocratically on a C18 column, ionized using a positive ion atmospheric pressure electrospray ionization source and analysed using multiple reaction monitoring mode. The chromatographic run time was 2.5min per injection, with retention times of 1.45, 1.02 and 0.95 min for PIO, M-III and M-IV, respectively. The

calibration curves of pioglitazone, M-III and M-IV were well fit over the range of 0.5-2000ng/ml (r(2)>0.998759) by using a weighted (1/x(2)) quadratic regression. The method is simple, rapid and rugged, and has been applied successfully to sample analysis for clinical studies.

Yamashita K, Murakami H, et al estimated a high performance liquid chromatographic (HPLC) method for the simultaneous determination of pioglitazone and its metabolites (M-I to M-V) in human serum and urine was developed. The method for serum involved the solid-phase and liquid-liquid extraction. Urine with and without enzymatic hydrolysis using betaglucuronidase was treated with liquid –liquid extraction. The compounds in the extract were analyzed using HPLC with UV detection at 269nm. The detection limits of pioglitazone, M-I, M-II, M-III, M-IV, and M-V in serum were 0.01-0.05 micrograms/ml, those in urine were 0.3-0.5 micrograms/ml, respectively. The method was applied to the clinical trials of pioglitazone.

Isam Ismail Salem et al estimated a sensitive and specific high-performance liquid chromatography – electrospray ionization –tandem mass spectrometry (HPLC-ESI-MS-MS) method has been developed at our center for the determination of glimepiride in human plasma. The compounds were separated on a prepacked C18 column using a mixture of acetonitrile, methanol and ammonium acetate buffer as mobile phase. Linearity was established for the range of concentrations 5.0-500.0ng/ml with a coefficient of determination (r^2) of 0.9998. Accuracy for glimepiride ranged from

100.58 to 104.48% at low, mid and high levels. The intra-day precision was better than 12.24%. The lower limit of quantitation (LLOQ) was identifiable and reproducible at 5.0ng/ml with a precision of 7.96%.

Gomes-P; Sippel-J, Jablonski-A; Steppe-M et al developed a method for the determination of rosiglitazone (Avandia) in coated tablets is described Micellar electrokinetic chromatographic, and high performance liquid chromatographic methods are employed.

Radhakrishna-T, Satyanarayana-J; Satyanarayana-A et al estimated an isocratic reversed phase liquid chromatographic (RP-LC) method has been developed and subsequently validated for the determination of rosiglitazone and its related impurities. Separation was achieved with a Symmetry C18 column and sodium phosphate buffer (pH adjusted to 6.2): acetonitrile (50:50, v/v) as eluent, at a flow rate of 1.0ml/min. UV detection was performed at 245 run. The method is simple, rapid, selective and stability indicating. Indole was used as internal standard for the purpose of quantification of rosiglitazone. The described method is linear over a range of 0.45-10mug/ml for related impurities and 180-910mgu/ml for assay of rosiglitazone. The method precision for the determination of assay and related compounds was below 1.0 and 3.6% RSD, respectively. The means recoveries of impurities were found to be in the range of 95-102%. The percentage recoveries of Active Pharmaceutical Ingredient (API) from dosage forms ranged from 99.02 to 101.30.

Sripalakit P, Neamhom P, et al performed an analytical method based on high-performance liquid chromatography (HPLC) with ultraviolet detection (269nm) was developed for the determination of pioglitazone in human plasma. Rosiglitazone was used as an internal standard. Chromatographic separation was achieved with a reversed phase Apollo C18 column and a mobile phase of methanol acetonitrile mixed phosphate buffer (pH 2.6; 10mm) (40:12:48, v/v/v) with a flow rate of 1.2ml/min. The calibration curve was linear over the range of 50-2000ng/ml (r(2)>0.9987) and the lower limit of quantification was 50ng/ml. The method was validated with excellent sensitivity, accuracy, precision, recovery and stability.

Jaafar I, A.L.Tamini et al developed a thin-layer chromatographic behavior of new oral antidiabetic drugs, pioglitazone, rosiglitazone, and repaglinide has been investigated. Mobile phases comprising 1,4-dioxane with phosphate buffers were used. Then, a simple, rapid, and stability-indicating high performance thin-layer chromatographic method has been developed and validated for the quantitative determination of ioglitazone in tablets. Analysis was performed with 1,4-dioxane-phosphate buffer of pH 4.4 (5:5) as the mobile phase. Detection and quantification were performed by classical densitometry at the wavelength of maximum absorption of pioglitazone, 266nm. A calibration plot was constructed in the range of 0.4-2.4µg/10µl and was linear with a good correlation coefficient (r = 0.9957).

Precision was validated by replicate analyses of standard solutions, and accuracy by analysis of fortified samples.

Radhakrishna-T, Rao-DS, Reddy-Go et al performed high performance liquid chromatographic (HPLC) and Micellar Electrokinetic Chromatographic (MEKC) methods have been developed for the determination of pioglitazone, a new englycemic antidiabetic agent. The MEKC method was compared with HPLC method using a 5mum symmetry C18 column (250 x 4.6mm W.) eluted with a mobile phase consisting of a mixture of 50% (v/v) acetonitrile and 10mm potassium dihydrogen phosphate buffer, adjusting the pH to 6.0 with 0.1 M KOH. The HPLC method is capable of detecting all process related compounds, which comparison was made.

S.Vijaya Saradhi et al performed a reverse phase high pressure liquid chromatographic method has been described for the estimation of glipizide in its pharmaceutical formulations using C8 column. The mobile phase consisted of acetonitrile, methanol and buffer (7.0ml of triethyl amine in 1000ml of water, pH adjusted to 3.0 $\pm$ 0.1 with orthophisphoric acid) in the ration of 35:50. The detection was carried out at 230nm and the linearity was found to be in the range of 0.1 to 10µg/ml. The method is simple, precise, specific, less time consuming and accurate for the estimation of glipizide in pharmaceutical dosage forms.

Puranik M, Wadher S.J., et al performed two simple, accurate and reproducible spectrophotometric methods, requiring no prior separation, have been developed for the simultaneous estimation of metformin HCL (MET) and Rosiglitazone meleate (ROSI) in combined dosage form. The first method employs formation and solving of simultaneous equations using 237 and 247nm as the two wavelength for forming equations. The second method is the absorption correction method using 237 and 283nm and methanol as solvent and linearity lies between 0-50mcg/ml. for metformin and Rosiglitazone at their respective wavelength.

S.B.Wankhede et al, developed a new validated reverse phase high performance liquid chromatographic method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet formulation. Chromatography was performed on a ODS Hypersil C18 (25cm x 4.6mm I.D) column from thermo in isocratic mode with mobile phase containing acetonitrile : 0.05 M KH_2PO_4pH3.0 (60:40). The flow rate was 1.0ml/min and the eluent was monitored at 271nm. The selected chromatographic conditions were found to effectively separate telmisartan (RT-5.19min) an dhydrochlorothiazide (RT-2.97min). Linearity for telmisartan and hydrochlorothiazide were found in the range of 4.1-20.48µg/ml and 1.28-6.4µg/ml, respectively. The proposed method was found to be accurate, precise, reproducible and specific and can be used for simultaneous analysis of these drugs in tablet formulation.

Giuseppe Carlucci et al developed a method for the simultaneous determination of losartan potassium and hydrochlorothiazide in tablets is described. The procedure, based on the use of reversed phase high performance liquid chromatography is linear in the concentration range 3.0-7.0μg/ml^{-1} for losartan and 0.5-2.0μg/ml^{-1} for hydrochlorothiazide, is simple and rapid and allows accurate and precise results. The limit of detection was 0.08μg/ml^{-1} for losartan and 0.05μg/ml^{-1} for hydrochlorothiazide.

Lande N.R., Shetkar B.M., Kadam S.S and **Dhaneshwar S.R** et al estimated two simple accurate and economical procedures for simultaneous estimation of Losartan Potassium and Hydrochlorothiazide in two component tablet formulations have been developed. The method employs simultaneous equations and Q-analysis. In all glass double distilled water Losartan Potassium has an absorbance maxima at 205nm, hydrochlorothiazide has three absorbance maximas at 225nm, 272nm and 315nm. Both drugs obey the Beer's Law in the concentration ranges employed for these methods. The results of analysis have been validated statistically and by recovery studies.

Nevin Erk et al performed a new simple, precise, rapid and selective reversed phase high performance liquid chromatographic (HPLC) and two spectrophotometric methods. The first method, is based on HPLC on a reversed phase column using a mobile phase 0.01N sodium dihydrogen phosphaye : methanol : acetonitrile (8:2:1 v/v/v) (pH 5.5) with detection at

265.0nm. The second method, is depend on ration derivative spectrophotometry, the amplitudes in the first derivative of the ration spectra at 238.360nm and at 230.423nm. The third method, based on compensation technique is presented for the derivative spectrophotometric determination of binary mixtures with overlapping spectra.

Farthing D, Fakhry I, et al performed a simple high performance liquid chromatographic (HPLC) method utilizing narrowbore chromatography was developed for the determination of hydrochlorothazide in human urine. A mobile phase of 0.1% aqueous acetic acid acetonitrile (93:7, v/v) pH 3 was used with a C18 analytical column and ultraviolet detection (UV). The method was utilized in a study evaluating if racial differences are present in the pharmacokinetic and pharmacodynamic effects of hydrochlorothiazide.

Wankhede SB, Tajne MR et al validated reverse phase high performance liquid chromatographic method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet formulation. Chromatography was performed on a ODS Hypersil C18 (25cm x 4.6mm I.D) column from thermo in isocratic mode with mobile phase containing acetonitrile: 0.05 M KH_2PO_4 pH3.0 (60:40). The flow rate was 1.0ml/min and the eluent was monitored at 271nm. The proposed method was found to be accurate, precise, reproducible and specific and can be used for simultaneous analysis of these drugs in tablet formulation.

Loannis Niopas et al developed a simple, specific, sensitive, precise, and accurate high performance liquid chromatography procedure was developed and validated for the analysis of hydrochlorothiazide in human plasma. Separated on a reversed phase column with acetonitrile/water 0:80, v/v as the eluent. Peaks were monitored at 271nm. The method was found to be linear in the 5 to 80ng/ml concentration range ($r<0.999$). The limit of quantitation was found to be 5ng/ml for 1ml plasma samples.

Nevin Erk et al estimated two new methods for the simultaneous determination of valsartan and hydrochlorothiazide in pharmaceutical dosage forms have been developed. The first method, based on compensation technique is presented for the derivative spectrophotometric determination of binary mixtures with overlapping spectra. By using ratios if the derivative maxima or the derivative minimum, the exact compensation of either component in the mixture can be achieved, followed by its determination. The second method, differential derivative spectrophotometry comprised of measurement of the difference absorptivities derivatized in the first order of a tablet extract in 0.1 N NaOH relative to that of an equimolar solution in methanol at wavelengths of 227.8 and 276.5nm, respectively. The proposed methods were accurate, sensitive, precise, reproducible and could be applied directly and easily to the pharmaceutical preparations.

Deanne L. Hertzog et al estimated a new class of potent angiotensin II receptor antagonists which are well-tolerated in the treatment of hypertension. A single method was developed to afford simultaneous quantitation of actives and degradates for each of the two existing formulations. Each method is presented herein and demonstrated to be suitable for quantitation to 0.1% levels of all relevant degradates, as well as 100% levels of respective drug substances.

A.G.Butterfield et al performed an aliquot of a tetrahydrofuran extract of the tablet, containing polythiazide as an internal standard, is chromatographed on a microparticulate silica gel column using a mobile phase of 0.01% (v/v) diethylamine, 5% (v/v) vhloroform, and 18% (v/v) 2-propanol in n-hexane.

Erturk S, Cetin SM, Atmaca S. et al developed two new simple and selective assay methods have been presented for the binary mixtures of moexipril hydrochloride (MOEX) and hydrochlorothiazide (HCTZ) in pharmaceutical formulations. The first method depends on second derivative ultraviolet spectrophotometry with zero-crossing measurements at 215 and 234nm for MOEX and HCTZ, respectively. The assay was linear over the concentration ranges 1.0-11.0 micro g ml(-1) for MOEX and 0.5-9.0 microg ml(-1) for HCTZ. The second method was based on isocratic reversed phase liquid chromatography by using a mobile phase acetonitrile-20mm phosphate buffer (pH 4.0) (50:50, v/v). Lisinopril was used as an internal

standard (IS) and the substances were detected at 212nm. The linearity range for both drugs was 0.5-12.0 microg ml(-1). The determination and detection limits were found to be 0.100 and 0.10 microg ml(-1) for MOEX and 0.025 and 0.005 microg ml(-1) for HCTZ, respectively.

Salem-H et al a new simple, precise, accurate and selective high performance thin layer chromatographic (HPTLC) method has been developed for the simultaneous determination of five mixtures: atenolol and chlorthalidone (Mix. I), enalapril maleate and hydrochlorothiazide (Mix. II), amiloride hydrochloride and hydrochlorothiazide (Mix. III), atenolol, chlorthalidone and amiloride hydrochloride (Mix. IV) and atenolol, hydrochlorothiazide and amiloride hydrochloride (Mix. V) in bulk powders and in pharmaceutical dosage forms.

Ferraro-MC, Castellano-PM, Kaufman-TS et al performed the development and validation of different chemometric methods such as classical least squares principal components regression and partial least squares with 1 dependent variable applied on UV spectral data and on their first derivatives for the simultaneous quantification of samples containing mixtures of amiloride hydrochloride, atenolol, hudrochlorothiazide and timolol maleate, is described.

Erk-N et al estimated two component mixtures of candesartan cilexetil (CAN) and hydrochlorothiazide (HYD) were assayed by first derivative and ratio derivative spectrophotometry. The first method depends

on zero-crossing and peak to base measurement. The first derivative amplitudes at 270.1 and 255.5nm were selected for the assay of (CAN) and (HYD), respectively. The second method depends on first derivative of the ratio spectra by division of the absorption spectrum of the binary mixture by a normalized spectrum of one of the components and then calculating the first derivative of the ratio spectrum.

Ertuerk-S, Cetin-SM; Atmaca-S et al performed the UV spectrophotometric and isocratic reversed phase liquid chromatography determination of binary mixtures of moexipril hydrochloride and hydrochlorothiazide are evaluated. The methods were successfully applied to the determination of moexipril hydrochloride and hydrochlorothiazide in synthetic mixtures and commercially available tablets with a high percentage recovery.

Erk-N, Ankara Univ, Fac Pharm et al developed the simultaneous determination of hydrochlorothiazide and irnesartan in a binary mixture without previous separation by three new analytical methods. The first method, based on compensation technique, is presented for the derivative spectrophotometric determination of binary mixtures with overlapping spectra. By using ratios of the derivative maxima or the derivative minimum, the exact contribution of either component in the binary mixture can be measured and the amounts quantified. The second method uses of the first derivative of the ratio spectra. The ratio spectra were obtained by dividing

the absorption spectra of the binary mixture by that of one of the components. The amplitudes in the first derivative of the ration spectra at 231, 266, 279, 238 and 248nm were selected to determine hydrochlorothiazide and irbesartan in binary mixtures.

Kamila MM, Mondal N, et al performed a simple, sensitive and accurate UV spectrophotometric method was developed for the assay of nebivolol hydrochloride in raw material and tablets. Validation of the method yielded good results concerning range, linearity, precision and accuracy the absorbance was measured at 282nm for nebivolol hydrochloride tablet solution. The linearity range was found to be 5-50microg/ml for the drug.

B,Dhandapani, et al developed a simple, fast, specific and precise High Performance Thin Layer Chromatographic Method (HPTLC) has been developed for estimation of Nebivolol hydrochloride in tablet dosage form. The stationary phase silica gel $G60F_{254}$ was selected for separation and the sample was developed using a mixture of Ethyl acetate : Methanol : Ammonia in the ratio 8.5:1:0.5v/v as mobile phase. Quantification was carried out at 285nm photometrically. The R_f value of Nebivolol hydrochloride was found to be 0.52 ± 0.02. Linearity was found to be in the concentration range of 250 to 1250ng/spot of Nebivolol.

Aboul-Enein HY, Ali I. et al performed studies on the effect of alcohols on the chiral discrimination mechanisms of amylase stationary phase on the enantioseparation of nebivolol by HPLC by achieving the enantiomeric resolution of (+/-)-nebivolol on Chiralpak AD and Chiralpak AD-RH columns with methanol, Ethanol, 1-propanol, 2-propnaol, 1-butanol as mobile phases at different flow rates. The (+)-RRRS enantiomer eluted first when using methanol, ethanol and 1-propanol, while the elution order was reversed when using 2-propanol and 1-butanol as the mobile phases. It has been concluded that the reversal elution order observed was due in part to the chiral cavities on the amylase CSP which were responsible for the bondings of different magnitude between chiral stationary phase and enantiomers.

Aboul-Enein HY, Ali I. et al performed HPLC enatiomeric resolution of nebivolol on normal and reversed amylase based chrial phases. The mobile phases used were pure ethanol and 1-propanol. The flow rates used were 0.5, 1.0 and 1.5ml/min. The best resolution was achieved at 0.5ml/min. flow rate with ethanol and 1-propanol on both Chiralpak AD and Chiralpak AD-RH stationary phases.

K.R.Gupta et al developed a HPTLC method was developed for analysis of above formulation. Atenolol and indapamide were separated on the plate coated with silica gel 60G F_{254} using a mixture of toluene : ethanol: acetone: acetic acid (7:2:5:3:0.3v/v) as mobile phase. The R_f value of

atenolol and indapamide was found to be 0.21 and 0.74, respectively. The percent recovery was found to be 99.2/100.51 and 99.07/98.65% for atenolol and indapamide by height by area, respectively.

Suraj P.Agarwal et al developed a new, simple, accurate and precise high performance thin layer chromatographic method has been developed for the estimation of artemether and arteether as pure drug powder and in pharmaceutical formulations. The method employs silica gel F_{254} as stationary phase on aluminium foil and mobile phase comprising toluene: butanol (10:1) in case of artemether and toluene : dichloromethane (0.5:10) for arteether. A solution of 10%v/v sulphuric acid in ethanol gave prominent well resolved blue coloured spot for artemether and vanillin in sulphuric acid in ethanolic solution gave prominent well-resolved pink coloured spot for arteether. The R_f values were 0.45 for artemether and 0.30 for arteether.

A.Annerberg et al performed a high throughout bioanalytical assay for the determination of lumefantrine in plasma has been developed and validated extensively. The within-day precisions for lumefantrine were 5.2, 3.5 and 2.5% at 200, 2000 and 15000ng/ml, respectively. The between day precisions were 4.0, 2.8 and 3.1% at 200, 2000 and 15000ng/ml, respectively. The lower limits of quantification (LLOQ) and the limits of detection (LOD) were 25 and 10ng/ml, respectively using 0.250ml plasma.

N.Lindegardh et al estimated a bioanalytical method for the determination of lumefantrine (LF) and its metabolite desbutyl-lumefantrine (DLF) in plasma by solid-phase extraction (SPE) and liquid chromatography has been developed. Plasma proteins were precipitated with acetonitrile : acetic acid (99:1, v/v). The compounds were analysed by liquid chromatography UV detection on a SB-CN (250mm x 4.6mm) column with a mobile phase containing acetonitrile sodium phosphate buffer pH (2.0; 0.1M) (55:45, v/v) and sodium perchlorate 0.05M. The limit of quantification was 0.024 and 0.021μg/ml for LF and DLF, respectively.

J.Karbwang, et al developed a rapid, selective, sensitive and reproducible HPLC with recutive electrochemical detection for quantitative determination of artemether (ART) and its plasma metabolite. Chromatographic separation was performed with a mobile phase of acetonitrile water (20:80, v/v) containing 0.1M acetic acid pH 5.0, running through a μBondapak CN column. The minimum detectable concentrations for ART and α-DHA in spiked plasma samples were 5 and 3ng/ml, respectively. The method was found to be suitable for use in clinical pharmacokinetic study.

S.S.Mohamed, S.A.Khalid, S.A., et al developed a sensitive, selective and reproducible GC-MS-SIM Method was developed for determination of artemether (ARM) and dihydroartemisinin (DHA) in plasma. The analysis was carried out using a HP-5MS 5%

phenylmethylsiloxane capillary column. The recoveries of ARM, DHA and ART were 94.9±1.6%, 92.2±4.1% and 81.3±1.2% respectively. The method is currently being used for pharmacokinetic studies.

N.Sandrenan, et al performed an analytical method for the determination of artemether (A) and its metabolite dihydroartemisinin (DHA) in human plasma has been developed and validated. The method is based on high-performance liquid chromatography (HPLC) and electrochemical detection in the reductive mode. Chromatography was performed on a Nova-Pak CN, 4μm analytical column (150mm x 3.9mm I.D.) at 35^0C. The mobile phase consisted of pH 5 acetate – acetonitrile (85:15, v/v) at a flow rate of 1ml/min. The mean recovery was 96% at the limit of quantitation (LOQ) of 10.9ng/ml with a CV of 13%.

B.M.kotecka, et al performed a comparison of bioassay and HPLC analysis of artesunate (ARTS) and dihydroartemisinin (DHA) in plasma. ARTS and DHA in plasma samples from patients treated with ARTS were quantifed any HPLC and expressed as DHA. Bioassay of active drug in plasma correlated well with specific chemical analysis by HPLC. ARTS and DHA appear to account for the total anatimalarial activity in plasma after ARTS administration.

Himanshu Naik, Daryl J. Murry, et al performed a sensitive method has been developed and validated for the determination of artesunate and its active metabolite dihydroartemisinin (DHA) in human plasma using artemisinin as an internal standard. Analysis was performed on a Shimadzu LCMS-2010. Chromatography was carried out using a Synergi Max-RP, 4µ, 75mm x 4.6mm column using glacial acetic acid 0.1%, acetonitrile and methanol mixture (38:46.5:15.5) as a mobile phase delivered at a flow rate of 0.5ml/min. The retention times of artesunate, α- and β- DHA and artemisinin were 17.4, 11.8, 18.7 and 13.4min, respectively.

C.Souppar et al developed a sensitive and selective method is described for the determination of artemether and its active dihydroiartemisinin metabolite in human plasma using artemisinin as internal standard. Chromatography was performed on C_{18} reversed-phase column using acetonitrile glacial acetic acid 0.1% (66:34) as a mobile phase. The method was fully validated over a concentration range of 5-200ng/ml using 0.5ml of human plasma per assay.

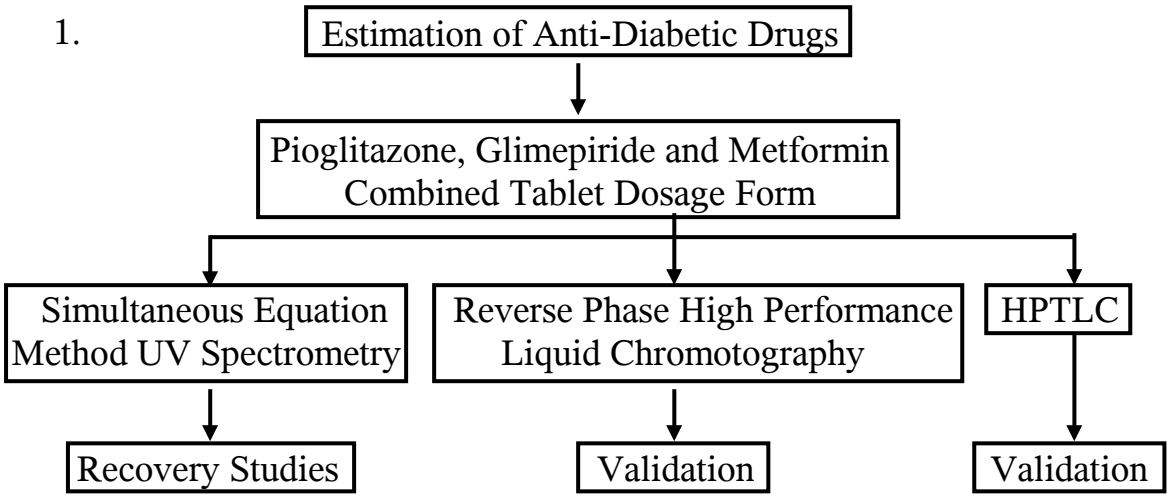
M.Rajanikanth, et al developed a sensitive and selective assay method for simultaneous determination of arteether and its metabolite dihydroartemisinin (DHA) in rat plasma by liquid chromatography mass spectrometry. Chromatographic separations were achieved by gradient elution of the analytes with an initial composition of methanol-potassium acetate buffer (pH 4; 73:27, v/v) to 100% methanol in 3min and maintained

for 5min on a Spheri-10, RP_{18} (100 x 4.6mm i.d.) column following an RP_{18} (30 x 4.6mm i.d.) guard column. Linearity in serum was observed over the range 4.375-70ng/ml for α–arteether and 10-160ng/ml for –arteether and DHA.

M.Bindschedler, et al estimated co-artemether is an oral tablet of artemether (20mg) and lumefantrine (120mg) for the treatment of falciparum malaria. Administration in the presence of mefloquine is likely, as co-artemether may be used following failure of antimalarial prophylaxis or treatment with mefloquine. The maximum and average QTc intervals for the first, third and sixth dosing intervals of co-artemether treatment were compared among treatments. Drug plasma concentrations were determined at identical times with the ECG recordings for exploratory pharmacokinetic / pharmacodynamic evaluation.

SCHEME OF WORK

1.



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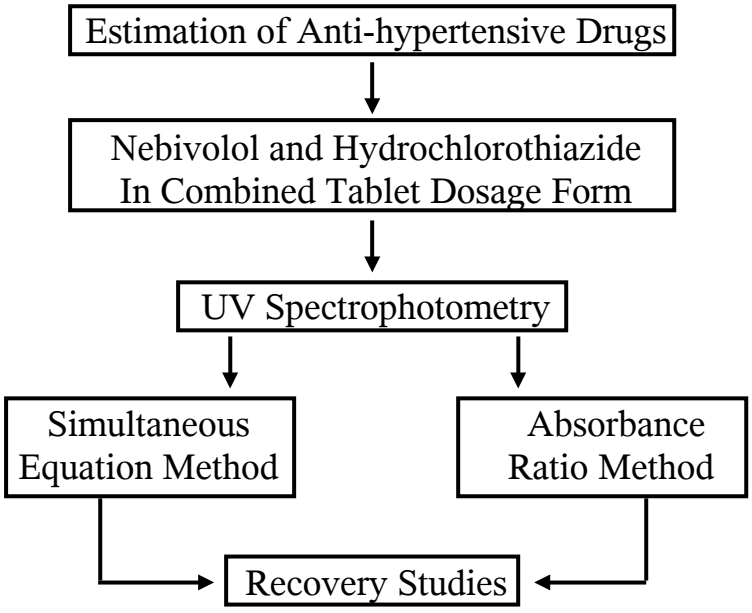

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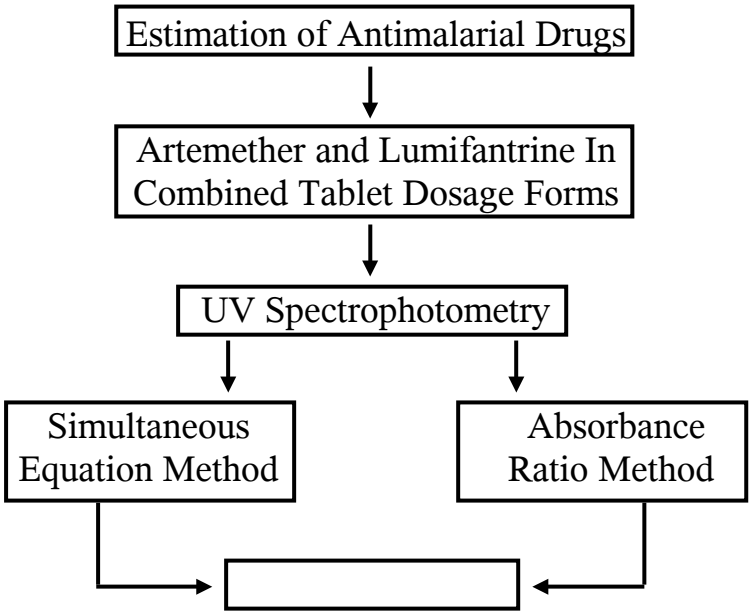

Recovery Studies

RESULTS AND DISCUSSION

Pioglitazone, Glimepiride and Metformin:

UV spectrophotometry by simultaneous equation method, RP-HPLC and HPTLC method were developed for analyzing Pioglitazone, Glimepiride and Metformin in combined tablet dosage form.

For UV spectrophotometry linearity was obtained in the concentration range of 5 to 50µg/ml for Glimepiride, 5 to 50µg/ml for Metformin and 5 to 40µg/ml for Pioglitazone. In quantitative determination the percentage Drug content was found to be 99.54%, 100.07% and 99.33% for Glimepiride, Metformin and Pioglitazone respectively. Recovery experiments were performed and it was within 98-102%, the percentage relative standard deviation were found to be <2% which shows high precision and accuracy of the method.

In HPLC method, HPLC conditions were optimized to obtain an adequate separation of eluted compounds. Initially various mobile phase were tried, to separate drugs. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, etc). The system with buffer (potassium dihydrogen ortho phosphate pH 6): Acetonitile : methanol (20:70:10v/v) with 0.7ml/min flow rate is quite robust. The optimum wavelength for detection was 240nm at which better detector response for

drugs was obtained. The average retention time for metformin, glimepiride and pioglitazone is 3.56, 8.61 and 9.52 min respectively.

According to USP system suitability test are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stick solution. The parameters are shown in table.

The calibration curve was found to be linear for the three drugs namely, Metformin, Pioglitazone and Glimepiride.

The low values of % Relative Standard Deviation indicate the method is precise and accurate. The developed method was very specific without the interference of excipients.

The percentage purity was 99.8%, 100.35% and 100.26% for Metformin, Glimepiride and Pioglitazone respectively.

The mean recoveries were found to be in the range of 98% to 102%.

Limit of detection for Metformin, Glimepiride and Pioglitazone was found to be 32.98µg/ml, 0.1607µg/ml and 0.1322µg/ml respectively.

Limit of quantitation for Metformin, Glimepiride and Pioglitazone was found to be 99.36µg/ml, 0.4871µg/ml and 0.4007µg/ml respectively.

Robustness of the proposed method was determined by changing the pH and flow rate.

Ruggedness of proposed method was determined by analysis of aliquots from homogenous slot by different analyst in different days using similar operational environmental condition. The result were within 98-102%.

In HPTLC method, HPTLC conditions were optimized to obtain an adequate separation of eluted compounds. The mobile system (ethanol : ammonia 8:2) with 0.4µl/spot. The average R_f value for metformin, pioglitazone and glimepiride is 0.13, 0.44 and 0.61 respectively.

The calibration curve was found to be linear for the three drugs namely, metformin, pioglitazone and glimepiride.

The low values of % RELATIVE STANDARD DEVIATION indicate the method is precise and accurate. The developed method was very specific without the interference of excipients.

The percentage purity was 98.9%, 100.15% and 100.24% for metformin, pioglitazone and glimepiride respectively.

The mean recoveries were found to be in the range of 98% to 102%.

Limit of detection for Metformin, Glimepiride and Pioglitazone was found to be 83.61mcg/ml, 1.48mcg/ml and 1.46mcg/ml respectively.

Limit of quantitation for Metformin, Glimepiride and Pioglitazone was found to be 253.38mcg/ml, 4.49mcg/ml and 4.5mcg/ml respectively.

Nebivolol and Hydrochlorothiazide:

Nebivolol and Hydrochlorothiazide in combined dosage form was estimated by simultaneous equation method and absorbance ratio method.

Linearity was obtained in the concentration range of 10µg/ml to 100µg/ml for nebivolol and 10µg/ml to 110µg/ml for hydrochlorothiazide.

The percentage drug content was found to 99.2% for both the drugs using simultaneous equation method.

The percentage drug content using absorbance ratio method was found to be 100.11% and 100.05% for nebivolol and hydrochlorothiazide respectively.

The recovery experiments were also performed for both method which was within the range of 98-102% and showed <2% RELATIVE STANDARD DEVIATION. This shows the high precision & accuracy of the proposed methods.

Lumifantrine and Artemether:

Lumifantrine and Artemether was estimated by simultaneous equation method and absorbance ratio method using UV spectrophotometry.

The linearity was obtained in the concentration range of 5 to 50µg/ml and 5 to 40µg/ml for Artemether and Lumifantrine respectively.

The percentage drug content was found to be 99.9% and 99.8% for Artemether and Lumifantrine respectively by using simultaneous equation method.

The percentage drug content using absorbance ratio method was found to be 100.08% and 100.11% for Artemether and Lumifantrine respectively.

The recovery experiments were performed for both the methods, which were within 98-102% and showed <2% RELATIVE STANDARD DEVIATION. This shows the precision & accuracy of the method.

SUMMARY AND CONCLUSION

Pioglitazone, Glimepiride and Metformin available in combined dosage form were analysed by UV- spectrophotometric simultaneous equation method, reverse phase high performance liquid chromatography and high performance thin layer chromatography.

On comparing all the three methods reverse phase high performance liquid chromatography was found to be more accurate, simple and rapid than other two methods.

Nebivolol and Hydrochlorothiazide in combined tablet dosage form was estimated using UV spectophotometry employing simultaneous equation method and absorbance ratio method.

On comparing both the method absorbance ratio method was found to be more accurate than simultaneous equation method for routine simultaneous estimation of both the drugs. The values of standard deviation and relative standard deviation were found to be satisfactory.

Lumifentrine and Artemether available in combined tablet dosage form was also estimated using UV spectophotometry employing simultaneous equation method and absorbance ratio method.

On comparing both the method absorbance ratio method was found to be more accurate than simultaneous equation method for routine simultaneous estimation of both the drugs. The values of standard deviation and relative standard deviation were found to be satisfactory.

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